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AFLP Diversity within and among Hardinggrass Populations

M. A. Rouf Mian,* John C. Zwonitzer, Yiwu Chen, Malay C. Saha, and Andrew A. Hopkins

ABSTRACT

Little information is available on the genetic diversity of hardinggrass (*Phalaris aquatica* L.), a cool-season forage grass with potential for use in the southern Great Plains. The objective of this study was to determine the genetic diversity within and among 22 promising hardinggrass populations, including plant introductions (PIs), breeding populations, and one cultivar. Nine plants from each population (198 genotypes) were characterized with amplified fragment length polymorphism (AFLP) markers. Genotypes were evaluated with nine selective primer combinations of fluorescent-labeled *Pst*I and *Mse*I primers, producing a total of 961 useful AFLP fragments. A high degree of genetic diversity was found, with a greater proportion of the diversity within (74%) rather than among (26%) populations. Clustering of populations on the basis of UPGMA closely followed the geographic origin and breeding history of the populations. Selections led to genetic shifts between two breeding populations, HG PI C1 and HG PI C2, although genetic diversity changed little if at all within these populations on the basis of polymorphism information content (PIC) scores of 0.22 versus 0.19, respectively. Two Moroccan PIs (PI 240248 and PI 517026) represent a distinct germplasm source on the basis of their genetic distances from other populations in the study. Genetic diversity data from this study will be helpful in grouping these accessions for development of breeding populations and various research purposes.

Hardinggrass, a cool-season perennial, is native to the Mediterranean region (Carlson et al., 1996). It is commonly used as pasture forage in Australia and was introduced to the USA from Australia in 1914 (Magness et al., 1971). Hardinggrass is a long-lived bunchgrass with adaption to mild climates with winter rainfall and thrives best on heavy soils. It is the most widely adapted range grass in California but is grown only sparingly in other areas of the Southwest. Where adapted, forage yield is high and quality is good. Cool-season grasses are of interest in the southern Great Plains because they can produce valuable forage during the spring, fall, and early winter seasons in this region. Cool-season grasses generally have poor persistence in the southern Great Plains (Klages, 1929; Hopkins et al., 2003; Malinowski et al., 2003). However, accessions of several cool-season grasses, including hardinggrass, with promising levels of persistence have been identified (Hopkins and West, 2002; http://www.ars-grin.gov/cgi-bin/npgs/html/eval.pl?491887; verified 15 July 2005).

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Little information is available on the genetic diversity of hardinggrass accessions and cultivars. Hardinggrass is an out-crossing species; thus, determination of genetic diversity is complicated by the fact that genetic variation exists among as well as within populations (cultivars or accessions). For this reason, genetic diversity studies in out-crossing species have been traditionally conducted by separately profiling DNA from a number of individuals within each population (Huff et al., 1993; Huff, 1997; Ubi et al., 2003). Within and among population genetic diversity is then determined by analyzing DNA profiles of these individuals. Breeders can use within and among population genetic diversity information for identifying diverse parental lines (Mian et al., 2002), classifying germplasm (Ferdinandez and Coulman, 2004; Larson et al., 2003), and monitoring genetic shifts in populations (Fu et al., 2004).

Molecular markers provide an efficient tool for evaluation of genetic diversity in plants. Various types of molecular markers (RFLP, RAPD, SSRs, and AFLP) have been successfully used to assess genetic diversity in out-crossing forage and turf grass species (Xu et al., 1994; Sun et al., 1998; Kubik et al., 1999; Sun et al., 1999; Diaby and Casler, 2003; Ubi et al., 2003). Among the different marker systems available, AFLP markers are particularly suitable for genotypic evaluation of out-crossing grass species like hardinggrass. AFLP markers are highly reproducible with overall error rates of less than 2% (Vos et al., 1995; Tohme et al., 1996), amenable to automation for high-throughput genotyping, and anonymous, so they do not require any sequence information. AFLP markers have been successfully used to determine genetic diversity in many plant species including forage and turf grasses (Sharma et al., 1996; Mace et al., 1999; Pillay and Myers, 1999; Zhang et al., 1999; Roldan-Ruiz et al., 2000; Guthridge et al., 2001; Ubi et al., 2003). The objective of this study was to evaluate genetic diversity within and among 22 hardinggrass populations with potential for persistence in the southern Great Plains.

MATERIALS AND METHODS

The hardinggrass PIs chosen for this research were among those having the greatest persistence and vigor following a grazing tolerance evaluation conducted at Ardmore, OK, from 1999 to 2001 (http://www.ars-grin.gov/cgi-bin/npgs/html/eval.pl?491887; verified 15 July 2005). Plants of each PI were grown from seeds obtained directly from the NPGS. Data regarding

Abbreviations: AFLP, amplified fragment length polymorphism; AMOVA, Analysis of molecular variance; FID, Forage Improvement Division; MDS, multidimensional scaling; NPGS, USDA-National Plant Germplasm System; PI, plant introduction; PIC, polymorphism information content; RAPD, randomly amplified polymorphic DNA; RFLP, restriction fragment length polymorphism; SSR, simple sequence repeat; UPGMA, Unweighted Pair Group Method Using Arithmetic Averages.

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origins of populations were obtained from the NPGS GRIN system (http://www.ars-grin.gov/npgs/searchgrin.html; verified 15 July 2005), the Margot Forde Forage Germplasm Centre database (Warren Williams at the Margot Forde Forage Germplasm Centre, Palmerston North, New Zealand), and from the literature for PI 578785 (Pedersen et al., 1984) and 'Maru' (Rumball, 1980).

The population HG PI C1 was developed by intermating 31 plants that survived for more than a year in a field at Ardmore, OK, under heavy grazing pressure and droughty conditions. Origins of these plants with the number of plants selected from each accession in parenthesis are as follows: PI 174295 (1), PI 201945 (2), PI 240255 (4), PI 240261 (2), PI 240263 (3), PI 240264 (2), PI 240266 (4), PI 254904 (3), PI 284193 (1), PI 294260 (2), PI 294267 (3), PI 441192 (1), PI 442539 (1), PI 476287 (1), PI 547392 (1). None of these PIs are in common with the PIs used for AFLP screening in this study. Seeds of HG PI C1 were used to establish an 800-plant selection nursery at Ardmore, OK, in fall, 1999. The 18 most vigorous plants that survived heavy grazing pressure were selected and placed in replicated plots in isolation in 2001. Seeds from these plants were harvested to form HG PI C2. MIP C1 was developed by intermating the eight most vigorous Maru plants selected from seeded plots following severe drought at Iowa Park, TX, in 1998. Seeds of MIP C1 were used to establish a 2000 plant selection nursery at Ardmore, OK, in 1999. The 32 most vigorous plants that survived heavy grazing pressure were selected and placed in replicated plots in isolation in 2001, and seeds from these plants were harvested to form MIP C2. For the present research, Maru plants were grown from seeds obtained from AgResearch, New Zealand.

Nine randomly selected plants from each of the 22 populations (Table 1) were used to assess within and among populations genetic diversity. Approximately 200 mg of tissue from young leaves of each plant was collected in a 2.0-mL microcentrifuge tube, immediately frozen in liquid N, and ground to fine powder with a Mixer Mill Type MM 300 (RETSCH, Hann Germany). DNA was extracted with the DNeasy Plant Mini Kit (Qiagen) with following modifications: 500 μL of AP1 buffer, 5 μL of RNase A, 165 μL AP2 buffer, and 90 μL of AE buffer. A Hoefer Dyna Quant 200 DNA fluorometer (Amersham Biosciences, Piscataway, NJ) was used to quantify the DNA concentrations.

Table 1. Geographical origin or source of 22 hardinggrass populations surveyed for genetic diversity.

Population name	Population code	Origin	Description
HG PI C1	A	USA	FID† breeding population
HG PI C2	B	USA	FID breeding population
PI 350674	C	Greece‡	NPGS§ accession
PI 358324	D	Algeria	NPGS accession
MIP C1	E	USA	FID breeding population
MIP C2	F	USA	FID breeding population
PI 517026	G	Morocco	NPGS accession
PI 578785	H	USA	Alabama from 12 PI accessions
PI 240234	I	Algeria	NPGS accession
PI 240248	J	Morocco	NPGS accession
PI 306744	K	Greece	NPGS accession
PI 350672	L	Algeria‡	NPGS accession
PI 240224	M	Algeria	NPGS accession
PI 240226	N	Algeria	NPGS accession
PI 284217	O	Turkey‡	NPGS accession
PI 284239	P	Australia‡	NPGS accession
PI 306739	Q	Greece	NPGS accession
PI 598946	R	Italy	NPGS accession
PI 598950	S	Italy	NPGS accession
Maru	T	New Zealand¶	cultivar
PI 240238	U	Algeria	NPGS accession
PI 284224	V	Australia‡	NPGS accession

† Forage Improvement Division, The Samuel Roberts Noble Foundation.
‡ Accession donated to the USDA-National Plant Germplasm System from Australia.
§ USDA-National Plant Germplasm System.
¶ Selected from germplasm from Argentina.

The AFLP procedure was performed according to the protocol of Vos et al. (1995) with 1 μg DNA for restriction digests with *Pst*I and *Mse*I. Preselective amplification was performed with *Pst*I+A and *Mse*I+C primers and a PCR profile from Marques et al. (1998) with following modifications: 20 cycles of 94°C for 30 s, 56°C for 60 s, and 72°C for 60 s. Selective amplifications were done with different combinations of 6-Fam fluorescent labeled *Pst*I primers with three selective bases and *Mse*I primers with three or four selective bases (*Pst*I + 3/*Mse*I+3 or 4) (Table 2).

Selective PCR amplifications were performed according to a protocol by Remington et al. (1999). AFLP fragments were separated by size by means of an ABI 3100 Genetic Analyzer (ABI, Foster City, CA) with 50-cm capillaries, POP-6 polymer, and rhodamine X (ROX) labeled GS400HD internal size standard. Raw data were analyzed by GeneScan analysis software (version 3.7, Applied Biosystems, Foster City, CA) and resulting GeneScan trace files were imported into Genograher version 1.2 (http://www.umanitoba.ca/afs/plant_science/psgendb/doc/genographer/index.html; verified 25 July 2005). AFLP fragments between 50 and 400 bp were scored in Genographer as present (A) or absent (B). Scores were recoded (by changing an A to 1 and a B to 0) and formatted for analyses.

Similarity coefficients between each pair of genotypes was calculated from Jaccard's coefficient by the formula $S_{ij} = a/(a + b + c)$, where a is the number of common bands (1, 1); b is the number of bands present in the first entry and absent in the second (1, 0); c is the number of bands absent in the first entry and present in the second (0, 1). The 0, 0 matches were not counted as useful information because the lack of an AFLP band in two genotypes may not be due to a common evolutionary event. Genetic distances were calculated as Euclidean distance, $D_{ij} = (1 - S_{ij})^{1/2}$. The matrix of genetic distances was submitted to hierarchical procedures, Ward's minimum-variance methods (SAS Institute, 1999) to cluster the entries in each population. The matrix of genetic distances generated from Jaccard's genetic dissimilarity coefficient was subjected to multidimensional scaling (MDS) (Shepard, 1974) by the MDS procedure in PC SAS (SAS Institute, 1999). The ABSOLUTE option was used to maintain the scale of 0 and 1 for making interpretation and graphing easier.

AMOVA, the analysis of molecular variance procedure in ARLEQUIN version 2.000 (Schneider et al., 2000), was used

Table 2. AFLP primer combinations used to characterize the genetic diversity of 22 hardinggrass populations and the number of fragments produced.

AFLP primer combinations	Number of AFLP bands scored	Number of polymorphic bands
P-ACC/M-CGGA†	104	80
P-ACC/M-CGCT	108	92
P-AGG/M-CTG	114	102
P-AGG/M-CACG	110	98
P-AGG/M-CGGC	75	59
P-AGT/M-CGGA	79	72
P-AGT/M-CGGC	122	94
P-AGT/M-CAG	111	104
P-AGT/M-CTG	138	103
Total	961	804

† P = *Pst*I and M = *Mse*I restriction enzymes.

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to estimate the components of variance attributable to differences among populations and among individuals within populations on the basis of the Euclidean square distance. A nonparametric permutation procedure with 1023 permutations was used to test the significance of variance components associated with the different possible levels of genetic structure in this study (Excoffier et al., 1992). The pairwise genetic distance (F_{st}) values, a value of F statistic analogs computed from AMOVA, were used to compare genetic distances between any two populations (Schneider et al., 2000). To demonstrate the relationship between populations, a distance matrix generated from AMOVA was used as input to perform a cluster analysis with UPGMA procedure (SAS Institute, 1999). Gene diversity over loci, so called polymorphism information content (PIC) scores (Anderson et al., 1992), was determined by the formula, $\hat{H} = \left(\frac{n}{n-1}\right)\left(1 - \sum_{i=1}^{k} p_i^2\right)$ (Nei, 1987). Its sampling variance also was estimated as $V(\hat{H}) = \frac{2}{n(n-1)}\left\{2(n-2)\left[\sum_{i=1}^{k} p_i^3 - \left(\sum_{i=1}^{k} p_i^2\right)^2\right] + \sum_{i=1}^{k} p_i^2 - \left(\sum_{i=1}^{k} p_i^2\right)^2\right\}$, where n is the number of gene copies in the sample, k is the number of haplotypes, and p_i is the sample frequency of the ith haplotype.

RESULTS AND DISCUSSION

AFLP Marker Diversity

From nine AFLP primer combinations, 961 fragments were scored (Table 2) and used for analysis. The number of AFLP fragments scored per combination ranged from 75 bands (P-AGG/M-CGGC) to 138 bands (P-AGT/M-CTG) (Table 2). Eight hundred four fragments, out of a total 961 fragments, were polymorphic. The percentage of polymorphism (83.7%) is similar to 84% reported by Ubi et al. (2003) for rhodesgrass (*Chloris gayana* Kunth). Polymorphic fragments within a population ranged from 34% (342) for PI 240248 to 68% (544) for PI 350674 (Table 3). Genetic diversity for a specific locus can be measured by PIC scores. Higher PIC score indicates higher probability that polymorphism will exist at a specific locus between two plants within an accession. Because all AFLP fragments are either present or absent, 0.50 will be the highest PIC score for any fragment. PIC scores ranged from 0.13 for PI 240248 and PI 284217 to 0.30 for PI 350674 (Table 3), indicating that these accessions had the least and the most amount of within population genetic diversity, respectively. Selection for persistence appears to have had little impact on genetic diversity within populations, as shown by PIC scores of HG PI C1 (0.22 ± 0.12) versus HG PI C2 (0.19 ± 0.10) as well as Maru (0.17 ± 0.09) and its derivatives MIP C1 (0.17 ± 0.09) and MIP C2 (0.17 ± 0.09).

Table 3. Number of polymorphic sites, percentage of polymorphic markers detected, and polymorphism information content (PIC) scores for 22 hardinggrass populations determined on the basis of 961 AFLP markers and nine individuals of each population.

Population	Number of polymorphic sites	Percentage of polymorphic markers detected	PIC score and standard deviations
HG PI C1	530	64%	0.22 ± 0.12
HG PI C2	452	47%	0.19 ± 0.10
PI 350674	544	68%	0.30 ± 0.16
PI 358324	462	49%	0.19 ± 0.10
MIP C1	408	43%	0.17 ± 0.09
MIP C2	339	44%	0.17 ± 0.09
PI 517026	424	44%	0.17 ± 0.09
PI 578785	482	55%	0.21 ± 0.11
PI 240234	481	50%	0.19 ± 0.10
PI 240248	342	34%	0.13 ± 0.07
PI 306744	401	45%	0.17 ± 0.09
PI 350672	438	46%	0.17 ± 0.09
PI 240224	496	52%	0.21 ± 0.11
PI 240226	434	49%	0.19 ± 0.11
PI 284217	338	36%	0.13 ± 0.07
PI 284239	413	47%	0.18 ± 0.10
PI 306739	384	40%	0.16 ± 0.09
PI 598946	338	40%	0.15 ± 0.08
PI 598950	409	43%	0.17 ± 0.09
Maru	412	43%	0.17 ± 0.09
PI 240238	193	46%	0.17 ± 0.09
PI 284224	219	48%	0.18 ± 0.10

The pattern of divergence among populations was primarily attributable to differences in fragment frequencies, but unique polymorphic fragments were also found for some populations (Table 4). Two unique fragments, pagtmctg368 and paccmcgct323, were present only in PI 517026 and PI 598950, respectively. This contrasts with Ubi et al. (2003) who reported no unique bands among rhodesgrass populations. However, Ubi et al. (2003) analyzed 15 genotypes per accession, whereas, in this study, we have analyzed nine genotypes per accession. Further research would be required to confirm if these unique fragments are associated with some specific characters. Removing these unique fragments from the analyses, along with the 10 other fragments listed as high frequency fragments in Table 4, did not change the UPGMA cluster groupings.

AMOVA to Partition Genetic Variance among Populations

On the basis of the AMOVA analysis, the differences among populations were significant, but greater variation was found among individuals within populations (Table 5). The variance within populations accounted for 74.1% of the total variance, while among population variance contributed only 25.9% (Table 5). Similarly, Ubi et al. (2003) found 82% within population and 18% among populations variation in rhodesgrass. The greater genetic variation within rather than among populations is likely due to the out-crossing nature of hardinggrass, in which the degree of within population polymorphism would be expected to be large (Ubi et al., 2003; Caetano-Anolles, 1998). Similar results have been observed in other out-crossing species such as perennial ryegrass (*Lolium perenne* L.), meadow fescue (*Festuca pratensis* Huds.), orchardgrass (*Dactylis glomerata* L.), and rhodesgrass (Huff, 1997; Kölliker et al., 1998; Ubi et al., 2003)

Population pairwise comparisons of values of F_{st} were interpreted as standardized interpopulation distances between populations. The population pairwise distances ranged from 0.03 between PI 240224 (M) and PI 240226 (N) to 0.52 between PI 240248 (J) and PI 284217 (O) (Table 6). The former two populations were both collected from Algeria, whereas the latter two accessions originated from geographically distant countries, Morocco and Turkey. In general, PI 240248 (J), and to

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Table 4. AFLP fragments that were unique or present at a high frequency in a specific population, as determined on the basis of analysis of 22 hardinggrass populations using nine plants per population. The top number represents plants within the given population, and the bottom number represents plants out of the total of 198, displaying the AFLP fragment.

Populations/AFLP fragments	HG PI C1	PI 350674	PI 358324	MIP C1	PI 517026	PI 240234	PI 306744	PI 350672	PI 598946	PI 598950
pagtcggc315	6 7									
pagtmctg362	5 7									
paggmcacg65		5 7								
paccmcgct365			5 6							
pagtmctg345				4 6						
pagtmctg368					7 7					
paggmcacg216					6 8					
pagtmctg140						5 6				
paggmcggc370							5 7			
pagtcggc369								5 7		
paggmcacg332									6 7	
paccmcgct323										7 7

a lesser extent PI 517026 (G), both originating from Morocco, was considered most genetically distinct from other populations in this research, because only a few F_{st} values less than 0.30 were observed (Table 6). In contrast, F_{st} values of 0.20 or less were generally common among other populations.

Cluster Analyses among Populations

To clearly visualize the genetic relationship among populations, the values of F_{st} from Table 6 were submitted to hierarchical clustering by UPGMA. The cluster analysis primarily separated the populations in close correspondence to their geographical origins and breeding history (Fig. 1). Two major clusters were generated from UPGMA clustering procedure. PI 517026 (G) and PI 240248 (J), both originating from Morocco, formed the most distinct cluster separating from the other large group. Likewise, within the large group, five of six accessions from Algeria (PI 358324, PI 240234, PI 240224, PI 240226, and PI 350672), both from Italy (PI 598946 and PI 598950), and two of three accessions from Greece (PI 306744 and PI 306739) formed distinct subgroups. On the basis of this correspondence of geographical origin and clustering, it is conceivable that PI 284224, which was donated to the NPGS from Australia, traces its origin back to germplasm from Algeria. MIP C1 and MIP C2 from the FID breeding program also grouped together, and showed a close relationship with Maru, as would be expected on the basis of the breeding history of these populations. A genetic shift appears to have occurred between HG PI C1 and HG PI C2, as these two populations clustered in separate groups. This shift may be associated with selection for favorable alleles conditioning persistence, or perhaps resulted from a genetic drift. Interestingly, HG PI C2 and PI 578785 (also known as AU 1) grouped closely together. Both populations trace their origin to a large number of PIs (>10), although none of those PIs appears to be in common nor do they all originate from the same countries. However, germplasm from Iraq, Israel, Morocco, and Turkey contributed to HG PI C2 as well as AU1, and both populations were selected at comparable latitudes in the southern USA for persistence and vigor, all of which may help explain the observed genetic similarity.

Multidimensional Scaling for All Individuals

A plot of the first two dimensions of the multidimensional scaling (MDS) further illustrated genetic variation within and among the 22 hardinggrass populations (Fig. 2). The first and second dimensions accounted for 39 and 22% of the total variation, respectively. Although Ward's Minimum Variance Cluster analysis revealed several groupings (data not shown), the genotypes in general formed a large, dispersed swarm. Three plants from PI 350674 (C) grouped far from this main swarm, further illustrating the relatively greater genetic diversity present within this population. The outlying genotype from HG PI C1 (A) may be a consequence of the broad genetic base of this population.

Table 5. Analysis of molecular variance (AMOVA) for 198 genotypes from 22 hardinggrass populations based on 961 AFLP markers.

Source of Variation	df	Sum of squares	Variance components	Percentage of variation	*P* value†
Among populations	21	6 909	27.7	25.9	$P < 0.0001$
Within populations	176	13 966	79.4	74.1	$P < 0.0001$
Total	197	20 875	107.1		

† On the basis of 1023 permutations.

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Table 6. Pairwise comparisons among 22 cultivars determined on the basis of genetic distances between populations (F_{st} value)† for 198 individuals.

	A‡	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
B	0.18																				
C	0.22	0.22																			
D	0.22	0.17	0.23																		
E	0.23	0.14	0.29	0.23																	
F	0.22	0.12	0.28	0.21	0.05																
G	0.22	0.32	0.31	0.30	0.38	0.36															
H	0.15	0.11	0.22	0.17	0.13	0.12	0.24														
I	0.21	0.19	0.22	0.11	0.26	0.24	0.28	0.15													
J	0.29	0.40	0.36	0.38	0.47	0.45	0.27	0.32	0.34												
K	0.27	0.21	0.20	0.26	0.31	0.28	0.35	0.21	0.24	0.45											
L	0.27	0.27	0.26	0.18	0.33	0.31	0.32	0.24	0.15	0.39	0.28										
M	0.20	0.16	0.21	0.12	0.24	0.21	0.28	0.16	0.14	0.36	0.17	0.15									
N	0.23	0.20	0.22	0.12	0.27	0.24	0.31	0.19	0.16	0.40	0.21	0.16	0.03								
O	0.30	0.22	0.32	0.28	0.19	0.19	0.44	0.21	0.31	0.52	0.31	0.35	0.25	0.29							
P	0.23	0.15	0.24	0.22	0.20	0.18	0.34	0.12	0.23	0.41	0.20	0.26	0.17	0.20	0.21						
Q	0.29	0.24	0.24	0.29	0.32	0.30	0.39	0.22	0.28	0.47	0.12	0.31	0.21	0.23	0.33	0.18					
R	0.30	0.25	0.27	0.24	0.29	0.26	0.40	0.23	0.25	0.49	0.25	0.30	0.20	0.20	0.31	0.23	0.27				
S	0.28	0.21	0.27	0.22	0.24	0.22	0.39	0.20	0.24	0.48	0.24	0.26	0.17	0.18	0.26	0.19	0.25	0.11			
T	0.22	0.14	0.28	0.22	0.09	0.09	0.37	0.14	0.25	0.46	0.26	0.28	0.19	0.22	0.14	0.12	0.25	0.22	0.18		
U	0.22	0.29	0.27	0.22	0.35	0.33	0.34	0.25	0.22	0.40	0.32	0.22	0.20	0.21	0.40	0.32	0.37	0.34	0.33	0.33	
V	0.19	0.25	0.22	0.27	0.33	0.31	0.29	0.20	0.25	0.36	0.26	0.27	0.21	0.25	0.36	0.25	0.31	0.33	0.32	0.30	0.15

† All F_{st} values are significant at ≤0.05 probability level.
‡ The population codes A = HG PI C1, B = HG PI C2, C = PI 350674, D = PI 358324, E = MIP C1, F = MIP C2, G = PI 517026, H = PI 578785, I = PI 240234, J = PI 240248, K = PI 306744, L = PI 350672, M = PI 240224, N = PI 240226, O = PI 284217, P = PI 284239, Q = PI 306739, R = PI 598946, S = PI 598950, T = 'Maru', U = PI 240238, V = PI 284224.

In summary, this is the first report on molecular marker diversity in hardinggrass. Several breeding strategies can be derived from the results of our research. Substantial genetic variation that could be exploited for selection is present within these hardinggrass populations. Further, the evidence indicates that selection

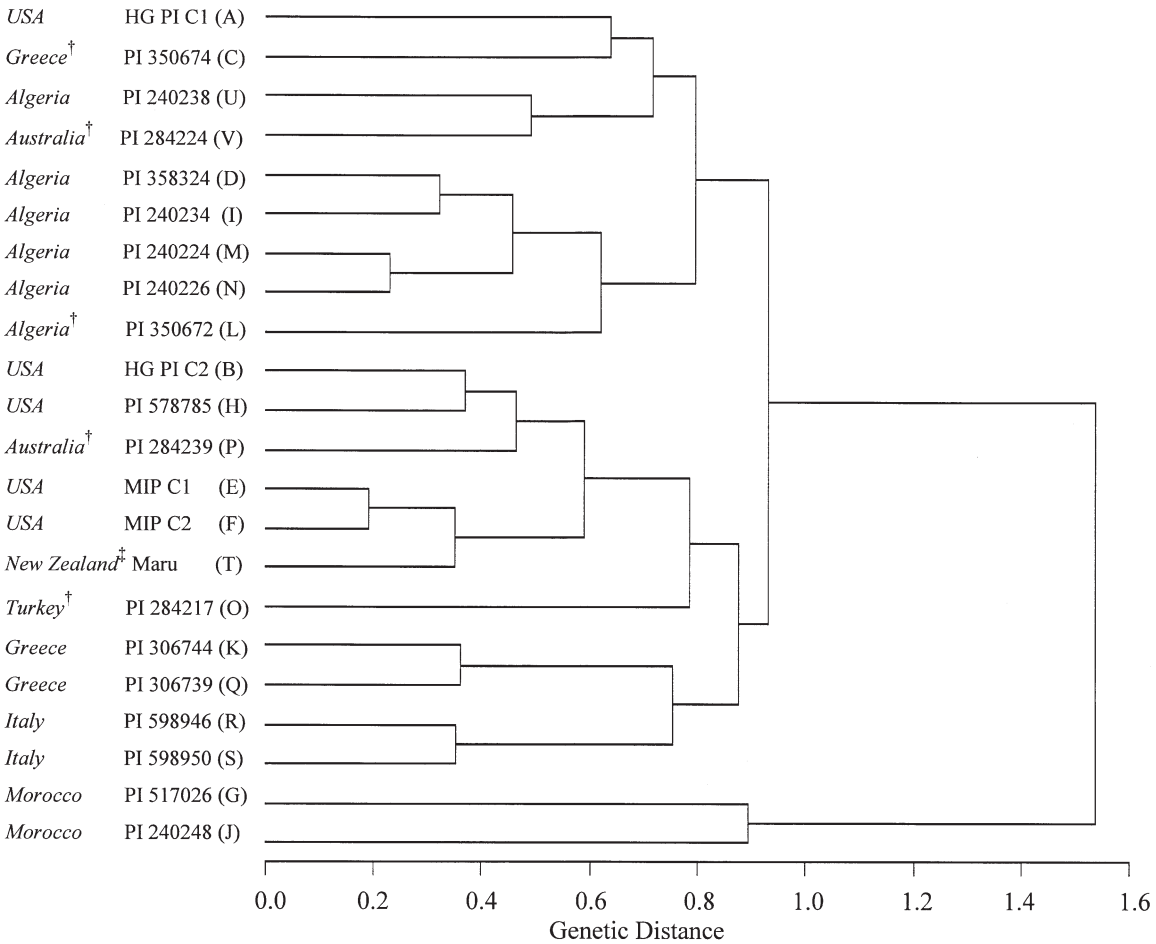

Fig. 1. UPGMA clustering of 22 hardinggrass populations using genetic distances generated from AMOVA. Origins of populations listed in italics. †Accession donated to the USDA-National Plant Germplasm System from Australia. ‡Selected from germplasm from Argentina.

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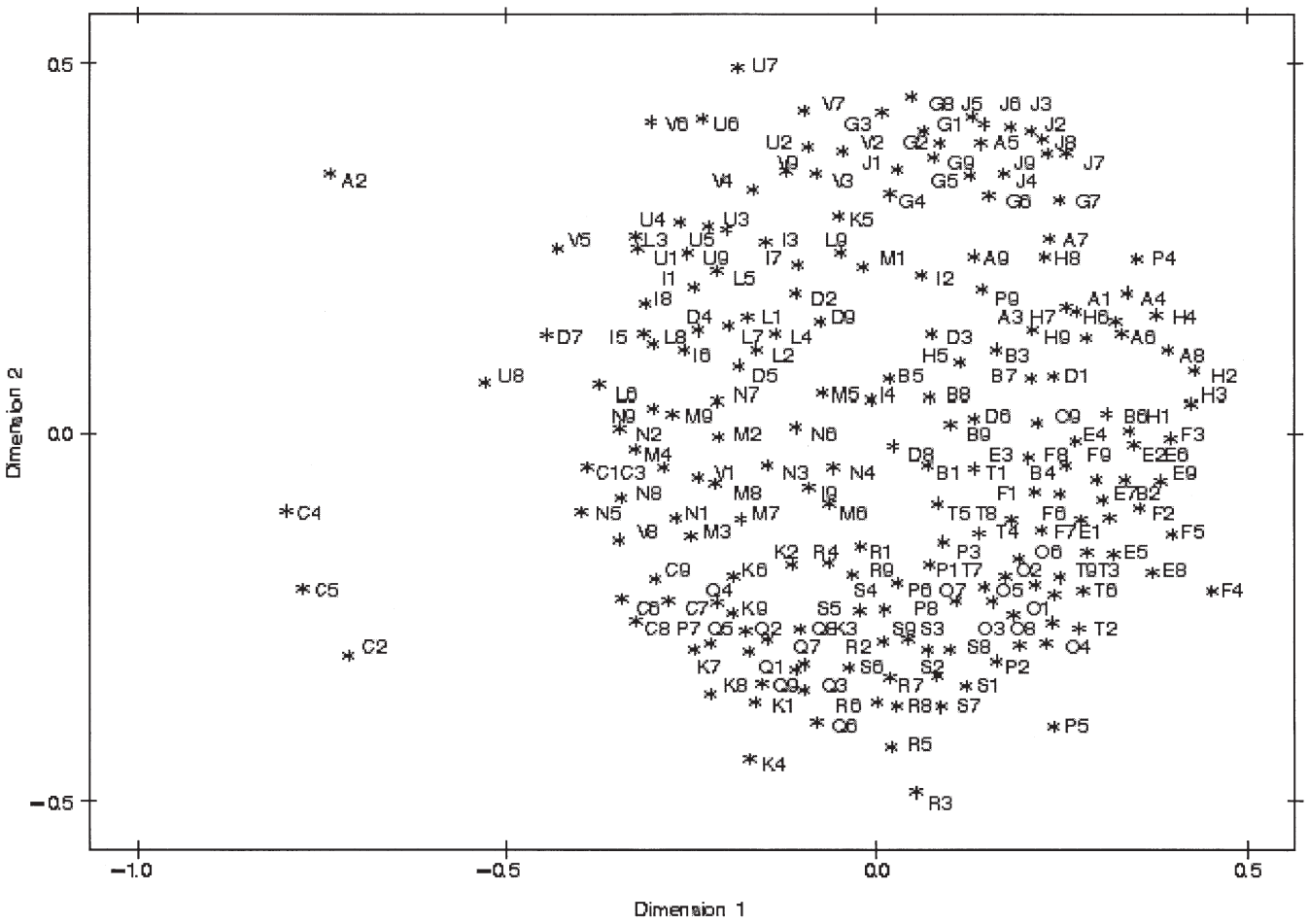

Fig. 2. Scatterplot based on the first two dimensions from multidimensional scaling analysis of AFLP fragment data demonstrating the genetic relationships among individuals of 22 populations of hardinggrass. Each of the nine individual genotypes within each population is represented by a number 1–9 preceded by the code name of the population. The population codes A = HG PI C1, B = HG PI C2, C = PI 350674, D = PI 358324, E = MIP C1, F = MIP C2, G = PI 517026, H = PI 578785, I = PI 240234, J = PI 240248, K = PI 306744, L = PI 350672, M = PI 240224, N = PI 240226, O = PI 284217, P = PI 284239, Q = PI 306739, R = PI 598946, S = PI 598950, T = 'Maru' U = PI 240238, V = PI 284224.

within hardinggrass populations need not result in a loss of genetic diversity. It is possible that more cycles of selection and/or a more stringent selection could result in significant loss of genetic diversity in these populations. The clustering of the accessions from Morocco away from other populations in the study suggests that these Moroccan populations represent distinct germplasm and could be used to construct distinctive populations. Broad based populations could be constructed as well from combinations of several of the non-Moroccan accessions. The FID Grass Breeding program is currently using both approaches to develop hardinggrass populations for further breeding and selection. Finally, the genetic differences between the Moroccan accessions and other populations in this research suggest a starting point for investigating possible heterosis in hardinggrass. Semihybrid cultivars might then be developed, as has been suggested for forage grasses (Brummer, 1999), to take advantage of heterosis.

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